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(71) Applicant *(for all designated States except US)*: KING'S COLLEGE LONDON [GB/GB]; Cornwall House, Waterloo Road, London SE1 8WA (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HOUGHTON, Peter, John [GB/GB]; 7 Culverden Road, London SE12 9LR (GB). WOLDERMARIAN, Tibebe, Zewdie [ET/GB]; Flat D, 188 Gloucester Terrace, London W2 6HT (GB). MAHMOOD, Naheed [GB/GB]; 156 Bromley Road, Beckenham, Kent BR3 2PG (GB).

(74) Agent: HOWARD, Paul, Nicholas; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

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(54) Title: ALKALOIDS AND THEIR ANTIVIRAL AGENTS

(57) Abstract

The present invention relates to chromone alkaloids isolated from the root, stem and root-bark of *Schumanniophyton magnificum* and *Schumanniophyton problematicum*, to analogues thereof and to therapeutic use thereof in the treatment of viral infection.

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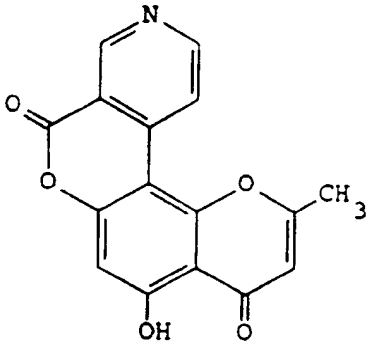
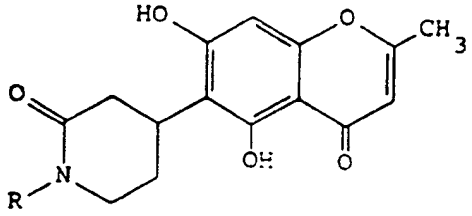
ALKALOIDS AND THEIR ANTIVIRAL AGENTS

The present invention relates to chromone alkaloids isolated from the root, stem and root-bark of *Schumanniophyton magnificum* and *S. problematicum*, which are trees found in West Central Africa. The present invention also relates to analogues of the alkaloids and to therapeutic uses of the alkaloids and their analogues. In particular, the invention relates to use of the alkaloids and their analogues in the prophylaxis and treatment of infection by human immunodeficiency virus (HIV), which is believed to be the aetiological agent in human acquired immunodeficiency syndrome (AIDS), and herpes simplex virus (HSV).

Chromone alkaloids were first isolated from *S. problematicum* by Schlittler *et al.* (Tetrahedron Letts., 2911-2914 (1978)), who reported three alkaloids, schumanniophytine (1) and two unnamed piperidin-2-ones (2a) and (2b):



(1)



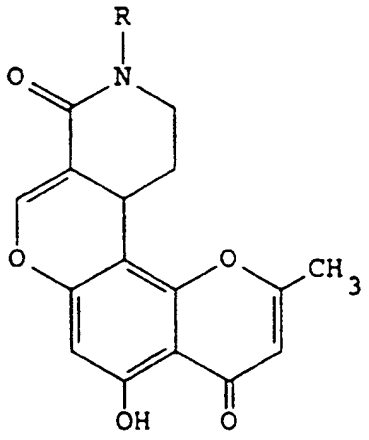
(2a) : R=H

(2b) ; R=Me

Further chromone alkaloids were isolated from *S. magnificum* Harms. (Rubiaceae) by Okogun *et al.* (Planta Medica, Journal of Medicinal Plant Research, 49, 95-98, (1983)), who

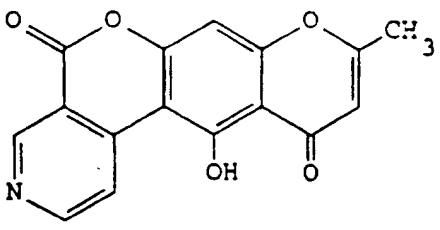
reported two alkaloids, schumannificine and N-methyl schumannificine, and the acetyl derivatives thereof which were prepared in the course of the isolation of the parent alkaloids.

Houghton *et al.* (Planta Medica, Journal of Medicinal Plant Research, 23-27, (1985); Ibid., 262-264 (1987); Ibid., 264-266 (1987); Ibid., 239-242 (1988); Phytochemical Analysis, 4, 9-13, (1993)) isolated a number of additional alkaloids including anhydroschumannificine (3), N-methyl anhydroschumannificine (3a), isoschumanniophytine (4) and rohitukine (5) and corrected the original structural formulae assignments of schumannificine 6a and N-methyl-schumannificine 6b. Structure 6a is now believed the correct structure of schumannificine. The compound is generally isolated as a mixture of 7'-isomers, although the isomers may be separated by HPLC. The stereochemistry at the 3' and 4' positions has not been determined.



(3a) : R=H

(3b) : R=Me



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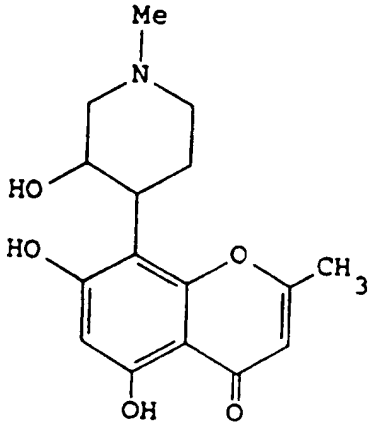

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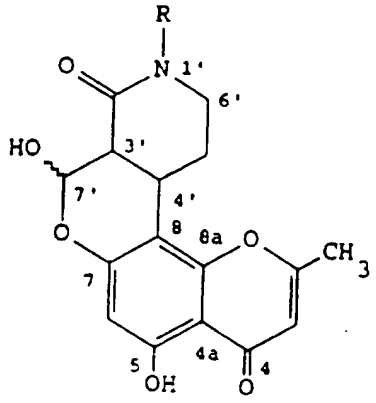


(6a) : R=H

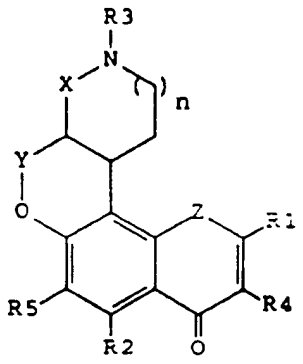
(6b) : R=Me

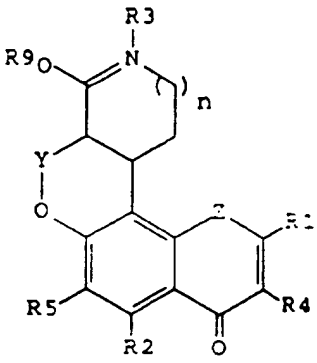
Rohitukine (5) has also been isolated from other plants belonging to the family Meliaceae. Rohitukine (5) and a number of derivatives thereof are reported (United States Patents 4,603,137 and 4,900,727 and Australian Patent Application AU-A-43841/89) to exhibit anti-inflammatory, analgesic, immuno-suppressive and anti-tumour activity.

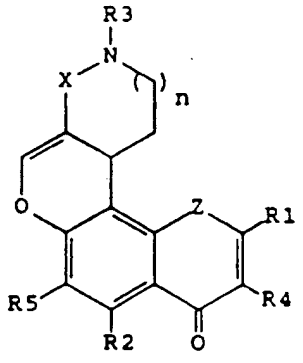
HIV is believed to be the aetiological agent in AIDS. (Barre-Sinoussi *et al.*, Science, 220, 868-870, (1983); Gallo *et al.*, Science, 224, 500-503, (1984)) and there are numerous reports of chemical compounds, such as AZT (zidovudine), possessing HIV-inhibitory activity. Such compounds, however, often exhibit problems with toxicity and other undesirable side effects in individual patients. There remains, therefore, the need for alternative and improved compounds for use in the prophylaxis and treatment of HIV infection.

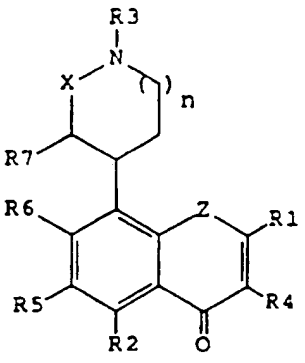
It has now been found that Schumanniophyton alkaloids and derivatives thereof are effective viral inhibitors whilst exhibiting low toxicity.

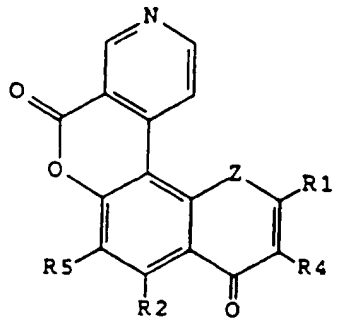
According to a first aspect of the present invention there is provided use of a compound of a formula selected from the group comprising:-

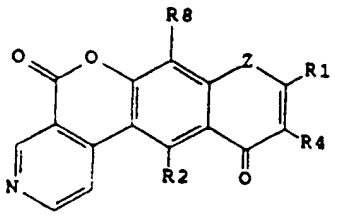
I 

II 

III 

IV 

V 

VI 

wherein

R^1, R^2, R^4, R^5, R^6, R^7 and R^8 may be the same or different and are selected from the group comprising hydrogen, hydroxy and substituted alkyl, alkoxy, alkoyloxy, aryl, aryloxy and aryloyloxy groups;

R^3 is selected from the group comprising hydrogen, carbohydrates and oligosaccharides, and substituted or unsubstituted alkyl, alkoyl, aryl and aryloyl groups;

R^9 is an alkyl group;

X is selected from $-CH_2-$ and $-C(O)-$;

Y is selected from $-CHR^{10}-$ and $-C(O)-$;

Z is selected from N and O;

n is selected from 0, 1 and 2;

R^{10} is selected from the group comprising hydrogen, hydroxy, carbohydrates and oligosaccharides, and substituted or unsubstituted alkyl, alkoxy, alkoyloxy, aryl, aryloxy and aryloyloxy groups;

and pharmaceutically acceptable derivatives thereof,

in the manufacture of a medicament for use in the treatment or prophylaxis of viral infection.

Preferably, the viral infection comprises HIV or HSV infection.

Pharmaceutically acceptable derivative means any pharmaceutically acceptable salt or addition compound or any

other compound which upon administration to the recipient is capable of providing (directly or indirectly) the parent compound or an anti-virally active metabolite or residue thereof.

Pharmaceutically acceptable salts include, for example, the hydrochloride, hydrobromide, sulphate, phosphate, acetate, oxalate, tartrate, citrate, maleate or fumarate. Pharmaceutically acceptable addition compounds include, for example, quaternary amines and esters of the compounds.

Reference to an alkyl group means a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g. alkenyl or alkynyl) hydrocarbyl radical. Where cyclic, the alkyl group is preferably C_3 to C_{12}, more preferably C_5 to C_{10}, more preferably C_5 to C_7. Where acyclic, the alkyl group is preferably C_1 to C_{10}, more preferably C_1 to C_6, more preferably methyl.

Reference to an aryl group means an aromatic group, such as phenyl or naphthyl, or a heteroaromatic group containing one or more, preferably one, heteratom, such as pyridyl, pyrrolyl, furanyl and thiophenyl. Preferably, the aryl group comprises phenyl.

The alkyl and aryl groups may be substituted or unsubstituted, preferably unsubstituted. Where substituted, there will generally be 1 to 3 substituents present, preferably 1 substituent. Substituents may include halogen atoms; oxygen containing groups such as oxo, hydroxy, carboxy, carboxyalkyl, alkoxy, alkoyl, alkoyloxy; nitrogen containing groups such as amino, alkylamino, dialkylamino, cyano, azide and nitro; sulphur containing groups such as thiol, alkylthiol, sulphonyl and sulphoxide; heterocyclic groups containing one or more, preferably one, heteroatom, such as thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl,

tetrahydrofuranyl, pyranyl, pyronyl, pyridyl, pyrazinyl, pyridazinyl, piperidyl, piperazinyl, morpholinyl, thionaphthyl, benzofuranyl, isobenzofuryl, indolyl, oxyindolyl, isoindolyl, indazolyl, indolinyl, 7-azaindolyl, isoindazolyl, benzopyranyl, coumarinyl, isocoumarinyl, quinolyl, isoquinolyl, naphthridinyl, cinnolinyl, quinazolinyl, pyridopyridyl, benzoxazinyl, quinoxadinyl, chromenyl, chromanyl, isochromanyl and carbolinyl; and aryl groups such as phenyl and substituted phenyl. Alkyl includes substituted and unsubstituted benzyl.

Reference to alkoxy means alkyl-O-. Reference to alkoyloxy means alkyl-C(O)-O-. Reference to aryloxy means aryl-O-. Reference to aryloyloxy means aryl-C(O)-O-.

Carbohydrates and oligosaccharides preferably comprise carbohydrates and oligosaccharides that improve the bioavailability of the compound, such as mono- up to penta-saccharides comprising, for example, glucose, glucuronic acid or rhamnose or their derivatives.

Preferably, R^1 is methyl or substituted or unsubstituted phenyl. More preferably, R^1 is methyl or unsubstituted phenyl, preferably methyl.

Preferably, R^2 is hydroxy or alkoyloxy. More preferably, R^2 is hydroxy.

Preferably, R^3 is hydrogen or alkyl. More preferably, R^3 is hydrogen.

Preferably, R^4 is hydrogen.

Preferably, R^5 is hydroxy or alkoyloxy. More preferably, R^5 is hydroxy.

Preferably, R^6 is hydroxy or alkoyloxy. More preferably, R^6 is hydroxy.

Preferably, R^7 is hydroxy or alkoyloxy.

Preferably, R^8 is hydrogen.

Preferably, R^{10} is hydroxy or alkoyloxy. More preferably, R^{10} is hydroxy.

With regard to compounds of formula I and III, X is preferably -C(O)-. With regard to compounds of formula IV, X is preferably $-CH_2-$.

Preferably, Y is $-CHR^{10}-$

Preferably, Z is O.

Preferably, n is 1

Preferably, R^4, R^5 and R^8 are hydrogen and Y is $-CHR^{10}-$.

Preferably, the compound is selected from the group comprising formulae I, II, III and IV, more preferably I and III, more preferably I.

Preferably, the compound is selected from the group comprising

Schumanniophytine
Isoschumanniophytine
N-methylschumanniophytine
Rohitukine
N-methylschumannificine
N-methylanhydroschumannificine
N-dimethylschumannificine
7'-(4-bromobenzoyl) N-methylschumannificine
Schumannificine
Anhydroschumannificine
N-demethyl-3'-acetyl-rohitukine
N,7'-diacetylschumannificine
N,7',5-triacetylschumannificine

7'-(4-bromobenzoyl)-schumannificine
7',5-di(4-bromobenzoyl)-schumannificine
7'-methoxyschumannificine
7',5-dimethoxyschumannificine

It will be appreciated that the compounds of the present invention exist in various diastereomeric and enantiomeric forms as a result of asymmetric centres in the compounds. The present invention incudes different diastereomers and enantiomers in isolation from each other, as well as mixtures.

The compounds of the present invention may be synthesised by conventional synthetic organic chemistry or may be prepared by isolation of the natural product from the root, stem or root-bark of *S. magnificum* or *S. problematicum* (Flora of West Tropical Africa, 2nd edition, (1963), ed. N. Hepper, volume II, pages 104-105 and 116, J. Hutchinson and J.M. Dalziel, pub. Crown Agents.) followed, where appropriate, by derivatisation using conventional synthetic organic chemistry.

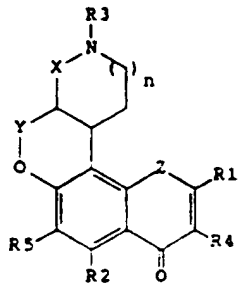
For example, compounds of formula II may be prepared from compounds of formula I (where X = -C(O)-) by treatment with BF_3 and R^9-O-R^9; compounds in which Y = -CH_2- may be prepared from the corresponding compounds in which Y = -CHOH- by tosylation and reduction; compounds in which Z = N can be prepared from the corresponding compounds in which Z = O by treatment with ammonia; compounds in which X = -CH_2- may be prepared from the corresponding compounds in which X = -C(O) by reduction with $LiAlH_4$; compounds in which Y = -C(O)- may be prepared from the corresponding compounds in which Y = -CHOH- by oxidation with Jones' reagent.

Whilst it is believed that the structural formulae assigned to the natural schumanniophyton alkaloids identified above are correct, and that the alkaloids have been purified to

homogeneity, it will be understood that the present invention extends to the natural product isolates described herein irrespective of the assigned formulae, to any co-isolates thereof and to derivatives thereof.

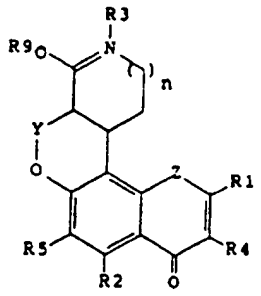
Accordingly, a further aspect of the present invention provides use of a schumanniophyton alkaloid or derivative thereof in the manufacture of a medicament for the treatment or prophylaxis of viral infection. A schumanniophyton alkaloid comprises an alkaloid isolatable from *S. magnificum* or *S. problematicum*. Derivatives thereof comprise alkaloids bearing alkyl, alkoxy, alkoyloxy, aryl, aryloxy and aryloyloxy substituents as defined hereinbefore.

According to a further aspect of the present invention there is provided a compound of a formula selected from the group comprising

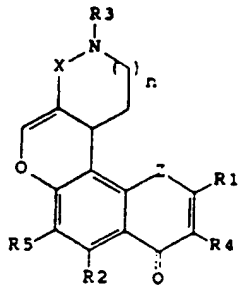
I 

wherein R^1-R^5, X, Y, Z and n are as defined above,

with the proviso that when R^1 is methyl, R^3 is hydrogen or methyl, R^4 is hydrogen, R^5 is hydrogen, X is -C(O)-, Y is CHR^{10}-, R^{10} is OH or OAc, Z is O and n is 1, then R^2 is not the same as R^{10};

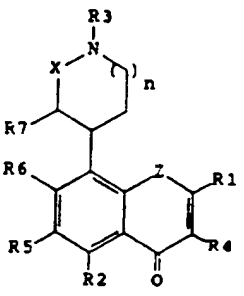
II 

wherein R^1-R^5, R^9, Y, Z and n are as defined above;

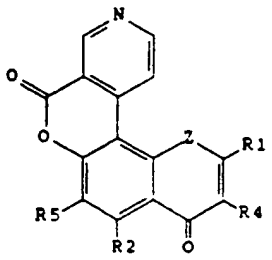
III 

wherein R^1-R^5, X, Z and n are as defined above,

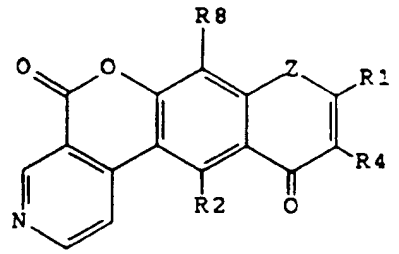
with the proviso that when R^1 is methyl, R^3 is hydrogen or methyl, R^4 is hydrogen, X is -C(O)-, Z is O and n is 1, then R^2 is not OH or OAc;

IV 

wherein R^1-R^7, X, Z and n are as defined above,
with the proviso that either or both X is -C(O)- and/or Z is N;

V 

wherein R^1, R^2, R^4, R^5 and Z are as defined above,
with the proviso that when R^1 is methyl, R^4 and R^5 are hydrogen and Z is O, then R^2 is not OH or OAc;

VI 

wherein R^1, R^2, R^4, R^8 and Z are as defined above,
with the proviso that when R^1 is methyl, R^4 and R^8 are hydrogen and Z is O then R^2 is not OH or OAc.

According to a further aspect of the present invention, there is provided a pharmaceutical composition for use in the treatment or prophylaxis of viral infection comprising a compound as hereinbefore defined in combination with a pharmaceutically acceptable excipient.

The compounds of the invention can be administered by oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal and topical administration.

For oral administration, the compounds of the invention will generally be provided in the form of tablets or capsules or as an aqueous solution or suspension.

Tablets for oral use may include the active ingredient mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

Capsules for oral use include hard gelatin capsules in which the active ingredient is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as

lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

According to a further aspect of the present invention there is provided a process for the production of a pharmaceutical composition comprising the step of combining a compound as hereinbefore defined with a pharmaceutically acceptable excipient.

According to a further aspect of the invention there is provided a method of treatment or prophylaxis of viral infection comprising administering to a patient in need of such treatment or prophylaxis an effective dose of a compound as hereinbefore defined.

The invention will now be described with reference to the following examples. It will be appreciated that what follows is by way of example only and that modifications to detail may be made whilst still falling within the scope of the invention.

EXPERIMENTAL

A. EXTRACTION AND ISOLATION OF ALKALOIDS FROM *SCHUMANNIOPHYTON MAGNIFICUM*

All solvents and reagents used were of AnalaR grade. Dried *S. magnificum* stem- and root-bark was obtained from Southeast Nigeria. Samples of dried stem- and root-bark of *S. problematicum* were obtained from Tiassle, Ivory Coast. Fresh *S. magnificum* stem and root material was collected from a forest reserve near Calabar and flown to London within 48 h where it was stored at -70°C. Reference vouchers are stored in the herbarium of the Chelsea Department of Pharmacy, King's College London.

500 g of powdered dried rootbark were extracted with hot methanol for 12 hours using a Soxhlet apparatus.

The extract was concentrated to a thick syrup and mixed with 2L chloroform:water 1:1. The two layers were separated using a separating funnel.

Chloroform layer

The chloroform layer was washed with 2 x 150mL water and then taken to dryness.

2.5g of the residue was adsorbed on to silica gel for Flash chromatography and fractionated on a silica column 1.5 x 25 cm. The following eluting solvents were used and 5ml aliquots collected.

Chloroform		250ml
Chloroform:methanol	19:1	500ml
Chloroform:methanol	9:1	500ml
Chloroform:methanol	6:1	500ml
Chloroform:methanol	3:1	500ml
Chloroform:methanol	1:1	500ml
Methanol		500ml

The fractions were screened for content using TLC (silica gel GF_{254}) chloroform:butanone 4:1 (system A), chloroform:methanol 12:1 (system B) and chloroform:methanol 6:1 (system C).

Developed plates were examined under UV (254nm and 365nm) light before spraying and in daylight after spraying with Dragendorff's reagent. Plates were then oversprayed with aqueous $Fe(III)Cl_3$ and the colours of zones noted.

Like fractions were bulked and taken to dryness. Compounds were extracted in the pure form by preparative TLC using systems A, B or C or butanone:methanol 12:1 (System D) and elution using methanol.

The compounds are eluted in the following order. (Details

of the R_f values, colour reactions and spectroscopic characteristics are given below).

Noreugenin (non-alkaloid)
N-methylanhydroschumannificine (3b)
Isoschumanniophytine (4)
Schumanniophytine (1)
N-methylschumannificine (6b)
Anhydroschumannifine (3a)
Schumannificine (6a)
Rohitukine (5)

Water Layer

Alkaloids were precipitated from the water layer with Dragendorff's reagent. The suspension was filtered and the residue dissolved in acetone:methanol:water 6:2:1. This solution was passed through an ion exchange column 15 x 1.5cm IRA 400 Cl^-. The eluate was concentrated and the residue separated by droplet counter-current chromatography butan-1-ol:methanol:water 5:1:5 using descending mode. Fractions (10mL) were collected and monitored on TLC (Silica gel/acetone:methanol:13.5M ammonia 4:1:1 - System E; detection as above for chloroform layer).

Similar fractions were bulked, concentrated and individual compounds extracted by prep TLC (silica gel GF_{254}/ethyl acetate:propan-2-ol:ammonia 65:35:10 - System F).

The two alkaloids isolated were (in order of elution):

N-methylschumanniophytine (7)
Rohitukine (5)

(Details of the R_f values, colour reactions and spectroscopic characteristics are given below).

Example 1

N-METHYLANHYDROSCHUMANNIFICINE (3b)

TLC behaviour

Colour:	
UV 254nm	Quenches
UV 365nm	No colour
After Dragendorff's	Brown
After $FeCl_3$	Dark Brown

R_f values

System A	0.28
System B	0.86
System D	0.45

Crystallisation

Could not obtain crystals

UV spectrum (Methanol; maxima nm (log ε))

224 (4.20), 246 (4.10), 256 (4.10), 310 (2.30)

IR spectrum (liquid paraffin)

1660 1620 1592

^1H NMR spectrum in $CDCl_3$ (δ ppm from TMS)

12.73 1*H* s(5-OH), 7.72 1*H* s (7'-H), 6.46 1*H* s (6-H), 6.12 1*H* s (3-H), 3.85 1*H* dd (4'-H), 3.6-3.0 4*H* m (5',6'-CH_2), 3.14 3*H* s (N-CH_3), 2.41 3 s (2-CH_3)

Mass spectrum

313 (25, M^+), 298(20), 192 (100)

Example 2

SCHUMANNIOPHYTINE (1)

TLC behaviour

Colour

UV 254nm	Pale yellow
UV 365nm	Lemon yellow
After Dragendorff's	Orange
After $FeCl_3$	Dark orange

R_f values

System A	0.09
System B	0.70
System D	0.06

Crystallisation

No crystals obtained

UV spectrum (Methanol; maxima nm (log ε))

225 (4.4), 237 (4.41), 251 (4.46), 256 (4.45), 292 (4.07), 318 (4.11)

IR spectrum (liquid paraffin)

3200-2400, 3070, 1750, 1660, 1620, 1580

^1H NMR spectrum in $CDCl_3$ (δ ppm from TMS)

13.47 1*H* bs (5-OH),, 9.58 1*H* s (2'-H), 8.97 1*H* d (6'-H), 8.48 1*H* d (5'-H), 6.82 1*H* s (6-H), 6.34 1*H* s (3-H), 2.66 3*H* s (2-CH_3)

Mass spectrum

295 (100, M^+), 267, 255 (18), 227

Example 3

ISOSCHUMANNIOPHYTINE (4)

TLC behaviour

Colour:

UV 254nm	Pale yellow
UV 365nm	Golden yellow
After Dragendorff's	Brown
After $FeCl_3$	Dark Brown

R_f values

System A	0.09
System B	0.72
System D	0.04

Crystallisation

No crystals could be obtained

UV spectrum (Methanol; maxima nm (log ϵ))

226 (4.11), 245 (3.96), 260 (3.96), 272 (3.56), 314 (2.98)

IR spectrum (liquid paraffin)

1750, 1660, 1620, 1590

^{1}NMR spectrum in $CDCl_3$ (δ ppm from TMS)

15.6 1*H* s (5-OH), 9.52 1*H* s (2'-H), 8.93 2*H* s (5',6'-H), 6.94 1*H* s (6-*H*), 6.24 1*H* s (3-H), 2.50 3*H* s (2-CH_3)

Mass spectrum

295 (100 M^+), 267 (12), 255 (15)

Example 4

N-METHYLSCHUMANNIFICINE (6b)

TLC behaviour

Colour:

UV 254nm	Quenches
UV 365nm	No colour
After Dragendorff's	Brown
After $FeCl_3$	Dark Brown

R_f values

System A	0.10
System B	0.72
System D	0.40

Crystallisation

Cream crystals from methanol MPt218-220°C

UV spectrum (Methanol; maxima nm (log ε))

220 (4.12), 225 (4.12), 253 (3.89), 260 (3.90), 277 (3.89), 290 (3.90), 310 (3.95), 320 (3.96)

IR spectrum (liquid paraffin)

3300, 1670, 1630, 1575

^{1}H NMR spectrum in $CDCl_3$ (δ ppm from TMS)

12.6 1*H* bs (5-OH), 6.87 1*H* bs (7'-OH), 6.35 1*H* s (6-H), 6.09 1*H* s (3-H), 5.60 1*H* d (7'-H), 3.7 1*H* m (4'-H), 3.3-3.1 3*H* m (5'-CH_2,3'-H), 3.05 3*H* s (N-CH_3), 2.65 1*H* m (6'-H), 2.39 3*H* s (2-CH_3), 2.20 1*H* m (6'-H)

Mass spectrum

331 (46, M^+), 313 (24), 205 (43), 192 (100)

Example 5

ANHYDROSCHUMANNIFICINE (3a)

TLC behaviour

Colour:

UV 254nm	Quenches
UV 365nm	No colour
After Dragendorff's	Blue
After $FeCl_3$	Blue-black

R_f values

System A	0.18
System B	0.79
System D	0.47

Crystallisation

No crystals could be obtained

UV spectrum (Methanol; maxima nm (log ϵ))

224 (4.08), 253 (4.32), 310 (2.40)

IR spectrum (liquid paraffin)

1670, 1640, 1620

^{1}H NMR spectrum in $CDCl_3$ (δ ppm from TMS)

12.7 1*H* s (5-OH), 7.77 1*H* dd (7'-H), 7.2 1*H* bs (NH), 6.27 1*H* s (6-H), 6.20 1*H* s (3-H), 4.00 1*H* dd (4'-H), 2.45 3*H* s (2-CH_3), 3.6-1.5 4 *H* m (5', 6'-CH_2)

Mass spectrum

299 (14, M^+), 192 (100)

Example 6

SCHUMANNIFICINE (6a)

TLC behaviour

Colour:

UV 254nm	Quenches
UV 365nm	No colour
After Dragendorff's	Pale orange
After $FeCl_3$	Blue-black

R_f values

System A	0.05
System B	0.37
System D	0.29

Crystallisation

Crystals from methanol MPt 244-246°C

UV spectrum (Methanol; maxima nm (log ε))

220 (4.13), 255 (4.13), 253 (3.86), 260 (3.88), 280 (3.88), 290 (3.85), 290 (3.85), 310 (3.95), 320 (3.96), 333 (3.97)

IR spectrum (liquid paraffin)

1650, 1620, 1165, 1090

^{1}H NMR spectrum in $CDCl_3$ (δ ppm from TMS)

12.6 1*H* bs (5-OH), 7.17 1*H* bs (N-H), 6.34 1*H* s (6-H), 6.11 1*H* s (3-H), 5.76 1*H* d (7'-H), 2.40 3*H* s (2-CH_3)

Mass spectrum

317 (21, M^+), 299 (15), 287 (17), 192 (100)

Example 7

ROHITUKINE (5)

TLC behaviour

Colour:

UV 254nm	Quenches
UV 365nm	No colour
After Dragendorff's	Pale orange
After $FeCl_3$	Blue-black

R_f values

System A	0.00
System E	0.95
System F	0.38

Crystallisation

Yellow crystals from absolute ethanol

UV spectrum (Methanol; maxima nm (log ε))

208 (4.37), 228 sh (4.12), 250 sh (3.97), 262 (4.10), 330 (3.68)

IR spectrum (liquid paraffin)

3400, 1660, 1612, 1560

^{1}H NMR spectrum in $CDCl_3$ (δ ppm from TMS)

6.79 1*H* s (6-H), 6.17 1*H* s (3-H), 4.44 1*H* d (3'-H), 3.63 1*H* dt (4'-H), 3.16-2.36 5*H* m (2', 5, 6'-H), 2.27 3*H* s (2-CH_3), 2.21 3*H* s (N-CH_3), 1.57 1*H* m (6'-H)

Mass spectrum

305 (M^+)

Example 8

N-METHYLSCHUMANNIOPHYTINE (7)

TLC behaviour

Colour:

Daylight	Bright yellow
UV 254nm	Yellow
UV 365nm	Bright yellow
After Dragendorff's	Orange
After $FeCl_3$	Dark orange

R_f values

System A	0.00
System E	0.75
System F	0.10

Crystallisation

Yellow crystals from absolute ethanol

UV spectrum (Methanol; maxima nm (log ϵ))

204 (4.69), 225 (4.4), 236 (4.39), 282 sh (4.07), 355 (4.12)

IR spectrum (liquid paraffin)

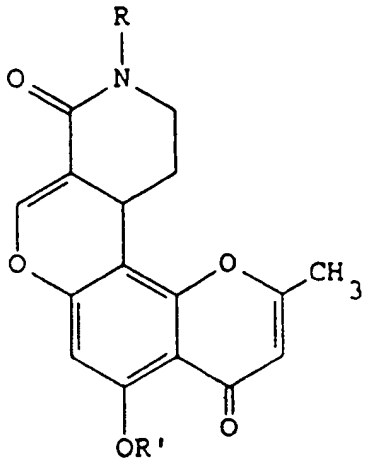
3200, 1750, 1660, 1620, 1585

^{1}H NMR spectrum in $CDCl_3$ (δ ppm from TMS)

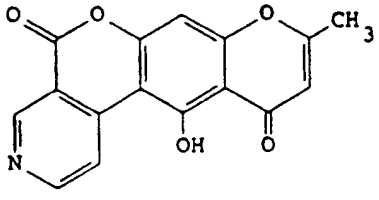
8.99 1*H* s (2'-H), 8.69 1H d (5'-H), 8.10 1*H* d (6'-H), 6.32 1*H* s (6-H), 6.10 1*H* s (3-H), 4.39 3*H* s (N-CH_3), 2.26 3*H* s (2-CH_3)

Mass spectrum

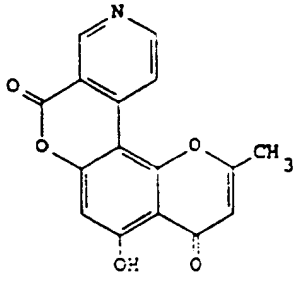
310 (5, M^+), 295 (20), 243 (76), 228 (56), 200 (32), 196 (20)



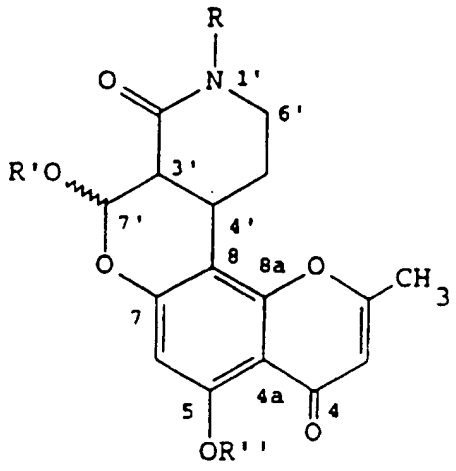
	R	R'
3a	H	H
3b	CH_3	H
3c	Ac	H
3d	Ac	Ac



(4)



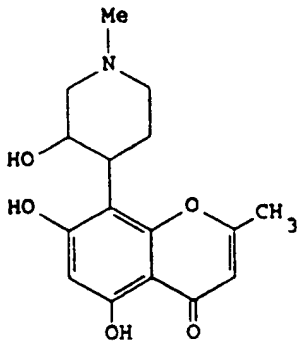
(1)



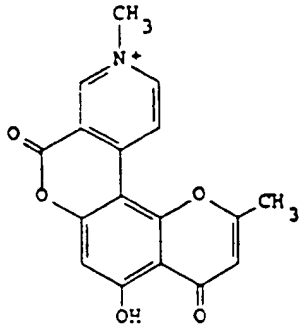
	R	R'	R"
6a	H	H	H
6b	CH_3	H	H
6c	H	BrBz	H
6d	H	BrBz	BrBz
6e	CH_3	BrBz	H
6f	Ac	Ac	H
6g	Ac	Ac	Ac
6h	CH_3	Ac	Ac
6j	H	Me	Me
6k	H	Me	H
6l	$(CH_3)_2$	H	H

Ac = CH_3CO-

BrBz = para-Bromo-Benzoyl



(5)



(7)

B. SYNTHESIS OF CHROMONE ALKALOID DERIVATIVES

Example 9

4-BROMOBENZOYL DERIVATIVES

60mg of the alkaloid was dissolved in 1mL pyridine. 60mg dimethylaminopyridine (DMAP) and 120 mg 4-bromo benzoyl chloride (4BrBzCl) were dissolved in 1mL dichloromethane and were added to the alkaloid solution. The solution was made up to 6mL with dichloromethane and kept at 25° for 72 hours.

The reaction mixture was poured into 20mL 1M $NaHCO_3$ and extracted with 3 x 10mL $CHCl_3$. The chloroform was washed with water and evaporated off under reduced pressure. The residue was examiend on TLC and the products isolated by prep TLC (silica gel/chloroform:methanol 12:1). The mass spectrum and ^1H NMR spectrum of each product was obtained.

Schumannificine when treated in this way gave two products

Example 9a

Di (4-bromobenzoyl) schumannificine (6d) R_f value 0.73

Mass spectrum

684 (15) M^+, 301 (100).

^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

8.04 (2H d J=8.6 3", 5"-H), 7.85 (2H, d J=8.6 Hz 3'5'-H), 7.85 (2H d J=8.6 2", 6"-H), 7.60 (2H, d J=8.6Hz 2', 6'-H), 7.17 (1H. d J=3.7Hz 7'-H), 6.76 (1H s 6-H), 6.01 (1H s 3-H), 4.82 (1H bs N-H) 3.89 (1H m 4'-H), 3.35 (1H m 6'-H), 3.24 (1H m 3'-H), 3.15 (1H m 6'-H), 2.80 (1H m 5'-H), 2.43 (3H s 2-CH_3), 2.25(1H m 5'-H).

Example 9b

Mono (4-bromobenzoyl) schumannificine (6c) R_f value 0.64

Mass spectrum

530 (40) M^+, 301 (100).

^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

12.7 (1H s 5-OH), 7.81 (2H, d J=8.6 Hz 3'5'-H), 7.54 (2H, d J=8.6Hz 2',6'-H), 7.14 (1H. d J=3.7Hz 7'-H), 6.38 (1H s 6-H), 6.12 (1H s 3-H), 4.82 (1H bs N-H) 3.82 (1H m 4'-H), 3.32 (1H m 6'-H), 3.18 (1H m 3'-H), 3.07 (1H m 6'-H), 2.80 (1H m 5'-H), 2.43 (3H s 2-CH_3), 2.19 (1H m 5'-H).

Example 9c

N-methylschumannificine gave one product
Mono (4-bromobenzoyl) N-methylschumannificine (6e) R_f value 0.69

Mass spectrum

514 (40) M^+, 331 (100).

^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

12.64 (1H s 5-OH), 7.84 (2H d J=8.6 Hz 3'5'-H), 7.54 (2H, d J=8.6Hz 2',6'-H), 7.17 (1H. d J=3.7Hz 7'-H), 6.38 (1H s 6-H), 6.12 (1H s 3-H), 3.81 (1H m 4'-H), 3.24-3.08 (3H m 3'-H, 6-CH_2), 2.92 (3H s N-CH_3), 2.81 (1H m 5'-H), 2.43 (3H s 2-CH_3), 2.22 (1H m 5'-H).

Example 10

ACETYLATION

Cold acetylation

60mg alkaloid is mixed with 1 ml pyridine and 2 ml acetic anhydride and kept for 72 hours at 25°. The solvents were evaporated under reduced pressure and the residue acidified with 1M HCl (20mL) and shaken with 3 x 10mL chloroform. The chloroform was evaporated to small volume and the products purified by prep TLC using silica gel/chloroform:methanol 12:1. The mass spectrum and ^1H NMR spectrum of each product was obtained.

Schumannificine treated in this way gave two products

Example 10a

N,5,7'-triacetylschumannificine (6g) R_f 0.67

Mass spectrum

443 (M^+ 20) 317 (100)

^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

2.09 (3H s 7'-acetate) 2.41 (3H s 7'-acetate) 2.41 (3H s 2-CH_3) 2.60 (3H s NAc) 6.12 (1H s 3-H) 6.62 (1H s 3-H) 6.95 (1H d 7'-H) 12.60 (3H s 5-OAc)

Example 10b

N,7'-diacetylschumannificine (6f) R_f 0.60

Mass spectrum

401 (M^+, 24), 359 (21), 341 (14), 317 (100)
^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

2.09 (s 3H 7'-acetate), 2.40 (s 3H 2-CH_3), 2.60 (s 3H N-Ac), 6.12 (s 1H 3-H), 6.62 (s 1H 3-H), 6.96 (d 1H 7'-H), 12.60 (3H s 5-OH)

N-methylschumannificine treated in this way gave two products

Example 10c

5,7'-diacetyl N-methylschumannificine (6h) R_f 0.75

Mass spectrum

415 (M^+, 24), 373 (18), 331 (100), 313 (22)

^1H NMR spectrum ($CDCl_3$ δ ppm from TMS)

2.08 (s 3H 7'-acetate), 2.36 (s 3H 5-acetate), 2.40 (s 3H 2-CH_3), 2.91 (s 3H N-CH_3), 6.04 (s 1H 3-H), 6.60 (s 1H 6-H), 6.97 (d 1H J = 4.0 7'-H)

Example 10d

7'-acetyl N-methylschumannificine (6j) R_f 0.66

Mass spectrum

363 (M^+ 30) 331 (100)

^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

2.08 (s 3H 7'-OAc), 2.40 (s 3H 2-CH_3), 2.91 (s 3H N-CH_3), 6.97 (1H d J=4 7'-H), 6.60 (1H s 6-H), 6.04 (1H s 3-H), 12.61 (1H s 5-OH)

Hot Acetylation

60mg alkaloid is mixed with 1 ml pyridine and 2 ml acetic anhydride and refluxed on a water bath for 3 hours. The solvents were evaporated under reduced pressure and the residue acidified with 1M HCl (20mL) and shaken with 3 x 10mL chloroform. The chloroform was evaporated to small volume and the products purified by prep TLC using silica gel/chloroform:methanol 12:1. The mass spectrum and ^1H NMR spectrum of each product was obtained.

Schumannificine treated in this way gave two dehydrated acetylated products

Example 10e

N,5,-diacetylanhydroschumannificine (3d) R_f 0.69

Mass spectrum

383 (28) M^+, 323 (45), 301 (51), 263 (100).

^{1}H NMR spectrum ($CDCl_3$) δ ppm from TMS

7.57 (1H d J=1.4 7'-H), 6.66 (1H s 6-H), 6.06 (1H s 3-H), 4.05 (1H dd J=8.2 J=2.2 6'-H), 3.95 (1H d J=3.8 4'-H), 3.68 (1H m 6'-H), 2.88 (1H m 5'-H), 2.62 (1H s N-OAc), 2.42 (3H s 2-CH_3), 2.36 (3H s 5-OAc), 1.94 (1H m 5'-H)

Example 10f

N-acetylanhydroschumannificine (3c) R_f 0.64

Mass spectrum

341 (60) M^+, 299 (100)

^{1}H NMR spectrum ($CDCl_3$) δ ppm from TMS

12.71 (1H s 5-OH), 7.57 (1H d J=1.4 7'-H), 6.43 (1H s 6-H), 6.15 (1H s 3-H), 4.04 (1H dd J=8.2 J=2.2 6'-H), 3.87 (1H d J=3.8 4'-H), 3.65 (1H m 6'-H), 2.89 (1H m 5'-H), 2.62 (1H s N-OAc), 2.42 (3H s 2-CH_3), 1.90 (1H m 5'-H).

Example 11

METHOXYLATION

30mg alkaloid was dissolved in 1ml 0.2m trimethylanilium hydroxide in methanol and refluxed for 1 hr. The solution was evaporated to dryness and acidified with 1M HCl and extracted with 3 x 15 mL chloroform.

The products were isolated using prep TLC (silica gel/chloroform:methanol 12:1) and the mass spectrum and ^{1}H NMR spectrum of each product was obtained.

Schumannificine gave two products

Example 11a

5,7'-dimethoxyschumannificine (6j) R_f 0.62

Mass spectrum

345 (100) M^+

^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

7.16 (1H bs NH), 6.38 (1H s 6-H), 6.09 (1H s 3-H), 5.57 (1H J= 4.0 7'-H), 3.81 (1H m 4'-H), 3.78 (3H s 5-CH_3), 3.76 (3H s 7'-OCH_3), 3.24-3.08 (3H m 3'-H, 6-CH_2), 2.81 (1H m 5'-H), 2.41 (3H s 2-CH_3), 2.22 (1H m 5'-H)

Example 11b

7'-methoxyschumannificine (6k) R_f 0.46

Mass spectrum

331 (100) M^+

^1H NMR spectrum ($CDCl_3$) δ ppm from TMS

12.68 (1H s 5-OH), 7.16 (1H bs NH), 6.38 (1H s 6-H), 6.09 (1H s 3-H), 5.57 (1H J=4.0 7'-H), 3.81 (1H m 4'-H), 3.76 (3H s 7'-OCH_3), 3.24-3.08 (3H m 3'-H, 6-CH_2), 2.81 (1H m 5'-H), 2.41 (3H s 2-CH_3), 2.22 (1H m 5'-H)

Example 12

FORMATION OF QUATERNARY AMINE FROM N-METHYLSCHUMANNIFICINE

20 mg N-methylschumannificine was refluxed with methyl iodide (5mL) for 30 min. The mixture was evaporated and the residue dissolved in 1 mL methanol. The product was purified by prep TLC (silica gel/ethyl acetate:propan-2-

ol:ammonia 65:35:10) and the mass spectrum and ^1H NMR spectrum obtained.

N-dimethylschumannificine (61)

Mass spectrum

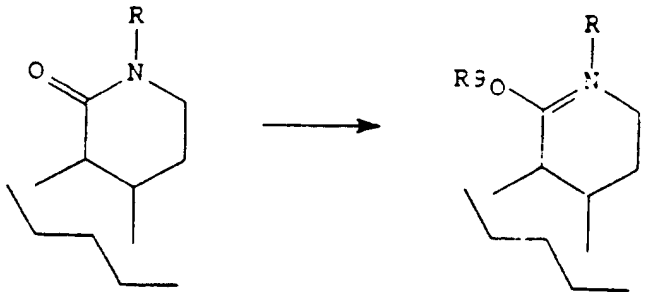
346 (100) M^+

^1H NMR spectrum (C_5D_5N) δ ppm from TMS

12.61 (1H s 5-OH), 6.78 (1H bs 7'-OH), 6.33 (1H s 6-H), 6.11 (1H s 3-H), 5.58 (1H d J=4.0 7'-H), 4.35 (6H s N-CH_3), 3.71 (1H m 4'-H), 3.31-3.10 (3H m 6'-CH_2, 3'-H), 2.65 (1H m 5-CH), 2.39 (3H s 2-CH_3), 2.21 (1H m 5-CH).

Example 13

FORMATION OF IMIDATES (Paquette, Kalihana, Hansen and Philips (1971) J. Am. Chem. Soc. 93 152.)

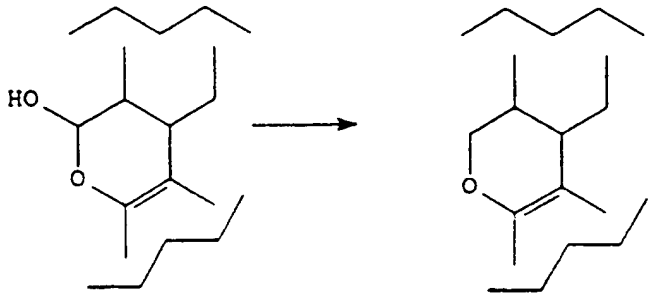


Boron trifluoride/ether reagent is dissolved in dry CH_2Cl_2 to form 0.27M solution. The alkaloid is added to the mixture which is then kept under nitrogen at 0°. 0.3M epichlorhydrin in CH_2Cl_2 is added dropwise and the mixture stirred continuously for 7 hours at 0°. Cold 5% aqueous

K_2CO_3 is then added to the mixture and after careful shaking the organic layer is removed, washed with water and the products isolated.

Example 14

REMOVAL OF HEMIACETAL OH BY TOSYLATION



The alkaloid is dissolved in dry benzene. *p*-Toluene sulphonic acid and dry $CaCl_2$ are added and the mixture refluxed for 3 hours.

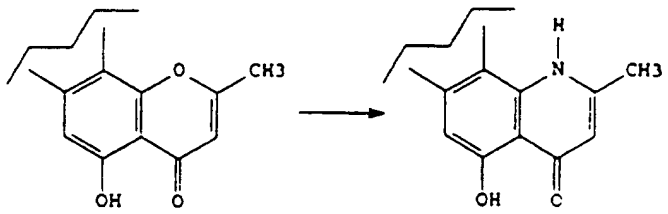
The benzene is washed with water and the alkaloid products are recovered from the benzene layer.

An alternative method

The alkaloid is dissolved in dry CH_2Cl_2 and equimolar *p*-toluene sulphonylhydrazine added. The mixture is refluxed at 100° for 30 min and after cooling, sodium borohydride is added. The reaction mixture is washed with water and the products recovered from the organic layer.

Example 15

REPLACEMENT OF O BY N IN THE CHROMONE RING

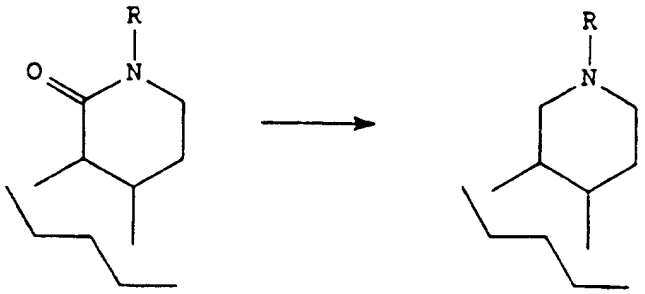


The alkaloid is dissolved in water:ethanol 1:1 and 10M NaOH added to make the mixture pH 12. 13.5M ammonia is then added to the mixture which is then refluxed at 100° for 30 min.

The pH of the reaction mixture is adjusted to 7 with dilute acid and CH_2Cl_2 is added to extract the products.

Example 16

REDUCTION OF THE PIPERIDINE RING (Weintraub, Oles and Kalish (1968) J. Org. Chem. 33 1679)



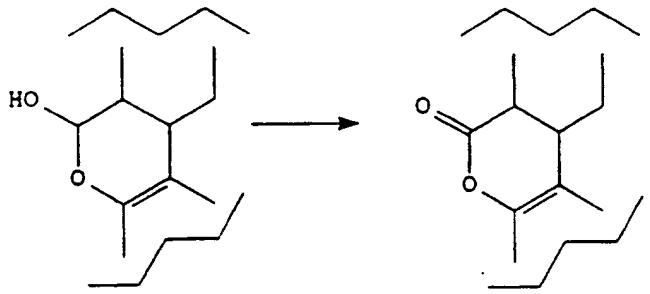
Boron trifluoride/ether reagent is dissolved in dry CH_2Cl_2

to form 0.27M solution. The alkaloid is added to the mixture which is then kept under nitrogen at 0°. 0.3M epichlorhydrin in Ch_2Cl_2 is added dropwise and the mixture stirred continuously for 7 hours at 0°. $LiBH_4$ is then added to the mixture which is then stirred at 0° for 3 hours.

Cold dilute acid is then added to the mixture and the alkaloid is recovered from the organic layer or by basification of the acid layer and extraction with CH_2Cl_2.

Example 17

OXIDATION OF THE HEMIACETAL TO A LACTONE (Bowden, Helibron, Jones and Weeden (1946) J. Chem. Soc. 39)



The alkaloid is dissolved in acetone and the solution maintained at 15°. Equimolar chromic acid solution is added dropwise with stirring. When all the chromic acid has been added sufficient CH_2Cl_2 is added to the mixture to form two layers. The products are recovered from the organic layer after washing.

C. BIOLOGICAL TESTING

The anti-HIV activity and toxicity of compounds were assessed in C8166 cells infected with HIV-1_{III-B}. Cells were grown in RMPI 1640 with 10% fetal calf serum. Forty-thousand cells per microtitre plate well were mixed with 5-fold dilutions of compound prior to addition of 10 $CCID_{50}$ (50% cell culture infectious dose) units of virus and incubated for 5-7 days. Formation of syncytia was examined from 2 days post-infection. Gp120 antigen produced at 5-7 days was measured by ELISA, using the lectin GNA (from Galanthus nivalis) to capture the glycoprotein and human anti-HIV serum for detection, as described by Mahmood and Hay, (J. Immunol. Methods, 151, 9-13, (1992)). Cell viability of virus-infected and uninfected control cells was measured by the MTT-Formazan method as described by Pauwels *et al.* (J. Virol. Meth., 20, 309-321 (1988)).

Results are presented in Table I.

The antiviral activity against herpes simplex type I (HSV-1, strain 17-1) was determined by measuring viral antigen produced in infected Vero or human lung embryonic cells MRC5 as described by Mahmood et al (Antiviral Chem. Chemother . 4, 235-240, (1993)). Five fold dilutions of compounds were added to duplicate cells just before adding virus at a multiplicity of infection of 0.01 plaque-forming units per cell. The cells were incubated 16-18h at 37° and then fixed with 3% formalin for 1-2h. Antigen was detected by ELISA using rabbit anti HSV-1 antibodies obtained from Dakopatts, Denmark. The cytotoxicity was assessed using the MTT-Formazan assay on growing Vero and human lung embryonic cells as described by Pauwels et al (J. Virol. Meth. 20, 309-321, (1988)).

Results are presented in Tables II and III.

TABLE I

COMPOUND	EC_{50} (μg/mL)	TC_{50} (μg/mL)
AZT (Control)	0.016	>1000
PYRIDINO-ALKALOIDS		
Schumanniophytine	8	100
Isoschumanniophytine	80	100
N-methylschumanniophytine	80	500
PIPERIDINO-ALKALOIDS		
Rohitukine	30	400
N-methylschumannificine	5	100
N-methylanhydroschumannificine	20	100
N-dimethylschumannificine	5	100
7'-(4-bromobenzoyl) N-methyl schumannificine	15	80
Schumannificine	1.6	100
Anhydroschumannificine	20	100
N-demethyl-3'-acetyl-rohitukine	--	500
N,7'-diacetylschumannificine	8	40
N,7',5-triacetyl-schumannificine	4	100
7'-(4-bromobenzoyl)-schumannificine	4	40
7',5-di(4-bromobenzoyl)-schumannificine	10	40
7'-methoxyschumannificine	5	250
7',5-dimethoxyschumannificine	40	400
CHROMONE (Control)		
Noreugenin	40	500

TABLE II

Anti-HSV activity of naturally-occurring chromone alkaloids

COMPOUND	EC_{50} (μM)	TC_{50} (μM) Vero	TC_{50} (μM) MRC5	Selectivity index TC_{50}/EC_{50} +
PIPERIDINO-ALKALOIDS				
Schumannificine	0.5	500	500	1000
N-methylschumannificine	0.5	>500	1000	>1000
Anhydroschumannificine	0.06	250	>500	>4000
N-methylanhydro-schumannificine	0.5	400	>250	800
Rohitukine	1.6	200	>100	12.5
N-demethylrohitukine acetate	100	>500	>1000	>10
PYRIDINO-ALKALOIDS				
Schumanniophytine	40	>500	500	>12.5
Isoschumanniophytine	50	>500	1000	>10
N-methylschumannio-phytine	50	>500	>1000	>10
CHROMONE				
Noreugenin	50	>500	1000	10

+ EC_{50} values for Vero cells

EC_{50} = the concentration of compound (μM) which reduced the production of viral antigen by 50%.

TC_{50} = the concentration of compound which reduced the viability of uninfected cells by 50% measured by the MTT-Formazan method

TABLE III

Anti-HSV activity of chromone alkaloid derivatives

	EC_{50} (μM)	TC_{50} (μM) Vero	TC_{50} (μM) MRCS	Selectivity index TC_{50}/EC_{50} +
COMPOUND				
7'-(4-bromobenzoyl) - schumannificine	0.4	400	500	1000
7',5-di(4-bromobenzoyl) schumannificine	0.4	>500	1000	1250
N,7'-diacetyl- schumannificine	0.1	>50	>100	>500
N,7',5-triacetyl- schumannificine	0.5	400	300	800
7'-butylschumannificine	0.4	100	ND	250
7',5-dimethoxy- schumannificine	0.2	250	1000	1250
7'-methoxy- schumannificine	15	400	500	26.7
N,N-dimethyl- schumannificine	15	300	>50	20

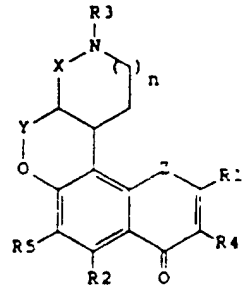
+ EC_{50} values for Vero cells

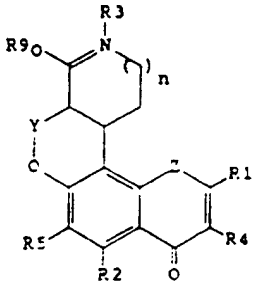
EC_{50} = the concentration of compound (μM) which reduced the production of viral antigen by 50%.

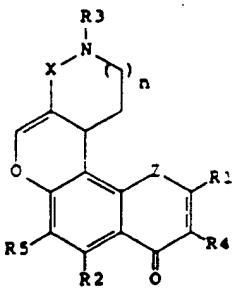
TC_{50} = the concentration of compound which reduced the viability of uninfected cells by 50% measured by the MTT-Formazan method

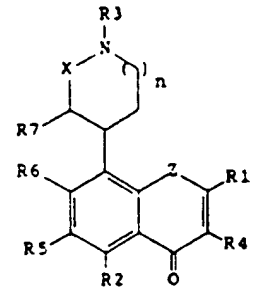
CLAIMS:

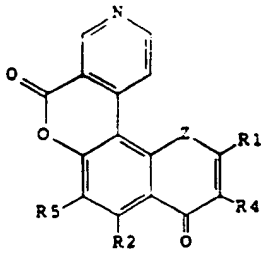
1. Use of a compound of a formula selected from the group comprising:-

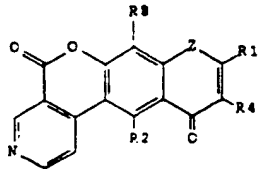
I 

II 

III 

IV 

V 

VI 

wherein

R^1, R^2, R^4, R^5, R^6, R^7 and R^8 may be the same of different and are selected from the group comprising hydrogen, hydroxy and substituted alkyl, alkoxy, alkoyloxy, aryl, aryloxy and aryloyloxy groups;

R^3 is selected from the group comprising hydrogen, carbohydrates and oligosaccharides, and substituted or

unsubstituted alkyl, alkoyl, aryl and aryloyl groups;
R^9 is an alkyl group;
X is selected from $-CH_2-$ and -C(O)-;
Y is selected from $-CHR^{10}-$ and -C(O)-;
Z is selected from N and O;
n is selected from 0, 1 and 2;
R^{10} is selected from the group comprising hydrogen, hydroxy, carbohydrates and oligosaccharides, and substituted or unsubstituted alkyl, alkoxy, alkoyloxy, aryl, aryloxy and aryloyloxy groups;
and pharmaceutically acceptable derivatives thereof, in the manufacture of a medicament for use in the treatment or prophylaxis of viral infection.

2. Use of a compound according to claim 1 wherein

R^4, R^5 and R^8 are hydrogen; and
Y is -CH(OH)-

3. Use of a compound according to claim 1 or 2 wherein

Z = O.

4. Use of a compound according to any preceding claim wherein the compound is of formula I or III.

5. Use of a compound according to any one of claims 1 to 3 wherein the compound is of formula V or VI.

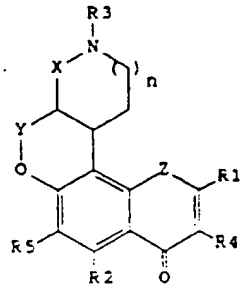
6. Use of a compound according to any preceding claim wherein the viral infection comprises HIV infection.

7. Use of a compound according to any one of claims 1 to 5 wherein the viral infection comprises HSV infection.

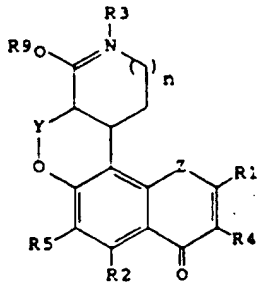
8. A pharmaceutical composition for use in the treatment or prophylaxis of viral infection comprising a compound as defined in any one of claims 1 to 5 in combination with a pharmaceutically acceptable excipient.

9. A method of treatment or prophylaxis of a viral infection comprising administering to a patient in need of such treatment or prophylaxis an effective dose of a compound as defined in any one of claims 1 to 5.

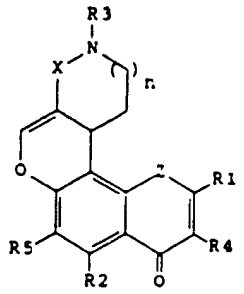
10. A compound of a formula selected from the group comprising

I 

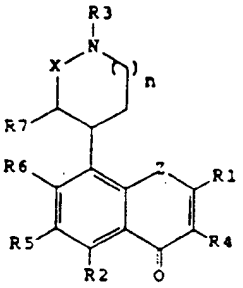
wherein R^1-R^5, X, Y, Z and n are as defined above,
with the proviso that when R^1 is methyl, R^3 is hydrogen or methyl, R^4 is hydrogen, R^5 is hydrogen, X is -C(O)-, Y is CHR^{10}-, R^{10} is OH or OAc, Z is O and n is 1, then R^2 is not the same as R^{10};

II 

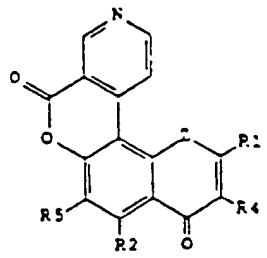
wherein R^1-R^5, R^9, Y, Z and n are as defined above;

III 

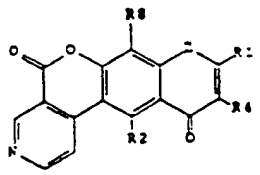
wherein R^1-R^5, X, Z and n are as defined above,
with the proviso that when R^1 is methyl, R^3 is hydrogen or methyl, R^4 is hydrogen, X is -C(O)-, Z is O and n is 1, then R^2 is not OH or OAc;

IV 

wherein R^1-R^7, X, Z and n are as defined above,
with the proviso that either or both X is -C(O)- and/or Z is N;

V 

wherein R^1, R^2, R^4, R^5 and Z are as defined above, with the proviso that when R^1 is methyl, R^4 and R^5 are hydrogen and Z is O, then R^2 is not OH or OAc;

VI 

wherein R^1, R^2, R^4, R^8 and Z are as defined above, with the proviso that when R^1 is methyl, R^4 and R^6 are hydrogen and Z is O, then R^2 is not OH or OAc.

INTERNATIONAL SEARCH REPORT

National Application No
PCT/GB 95/02091

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/435 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF PHARMACY AND PHARMACOLOGY, 46 (SUPPL. 2). 1994. 1061., HOUGHTON P J ET AL 'Antiviral activity of chromone alkaloids from Schumanniophyton magnificum' see the whole document ---	1-10
X	ANTIVIRAL RES., 1994, 235-44, HOUGHTON, P. J. ET AL 'Antiviral activity of natural and semi-synthetic chromone alkaloids' see the whole document ---	1-10
A	PLANTA MED, 53 (3). 1987. 264-266., HOUGHTON P J 'REVISION OF STRUCTURES OF SOME SCHUMANNIOPHYTON ALKALOIDS' see the whole document ---	1-10

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 1995	16.01.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Mair, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PLANTA MED, 0 (1). 1985. 23-27., HOUGHTON P J ET AL 'NOVEL CHROMONE ALKALOIDS FROM SCHUMANNIOPHYTON-MAGNIFICUM' see the whole document ---	9
A	ANTIVIRAL RESEARCH, vol. 22, no. 2-3, 1993 pages 189-199, MAHMOOD, N. ET AL 'Inhibiton of HIV infection by flavanoids' see the whole document ---	1-9
A	JOURNAL OF NATURAL PRODUCTS, vol. 55, no. 2, February 1992 pages 207-213, BEUTLER, J.A. ET AL 'Anti-HIV and cytotoxic alkaloids from Buchenavia Capitata' see the whole document especially page 211, line 23-25 -----	1-9

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB95/02091

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: ALL
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
In view of the large number of compounds which are theoretically defined by the formulae of claim 1 and 10, the search had to be restricted to the specifically exemplified compounds and the general concept of the application.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.